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Anesthetic Methods for the Moon Snail *Polinices lewisii*

by

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Abstract. Nine chemicals and hypothermia were tested on the moon snail *Polinices lewisii* for their effectiveness as anesthetics. Hypothermia and five of the drugs induced anesthesia. Althesin, a mixture of the steroids alphaxalone and alphadolone acetate, appeared to be the most desirable anesthetic. Hypothermia and xylazine can also be recommended for routine use. Less favored were ethanol and sodium pentobarbital. Benzyl alcohol was the most lethal of the compounds tested.

INTRODUCTION

SUCCESSFUL SURGERY, A central requirement for many physiological studies, often depends on an effective method of anesthesia. Although anesthetic procedures are well established for vertebrates, particularly mammals (GILMAN *et al.*, 1980), this is not the case with invertebrates despite the use of invertebrate preparations in studying the neuronal and synaptic activity of many mammalian anesthetics (JUDGE, 1980). In the mollusks only a few attempts have been made to systematically evaluate the effectiveness of anesthetic procedures (*e.g.*, ANDREWS & TANSEY, 1981 for a cephalopod; RUNHAM *et al.*, 1965, for several gastropods). Although RUNHAM *et al.* (1965) found several agents to be effective anesthetics, most of these suffered the drawback of exceedingly long induction times. This study, an attempt to update the RUNHAM *et al.* (1965) investigation, details the search for an effective, quick-acting anesthetic for the moon snail *Polinices lewisii* (Gould, 1847).

MATERIALS AND METHODS

The moon snails used in this study ranged in wet weight from 75 to 160 g; animals were weighed in the retracted state. All animals, collected in Barkley Sound, Vancouver Island, were held for at least five days in running seawater at 31 to 32‰ salinity and 10 to 12°C before experimentation was commenced. The holding tank was provided with a fine sandy bottom.

The anesthetic methods tried can be divided into two categories: physical and chemical. In the physical method, hypothermia, an animal was placed in a seawater bath and the bath temperature dropped to 2°C over a one-hour period. In the chemical methods, the following agents were tried: Althesin (Galaxo Laboratories), benzyl alcohol, carbon dioxide, ethanol, magnesium chloride, MS 222 (Sigma), quinaldine, sodium pentobarbital (Somnotol-MTC Pharmaceuticals), and xylazine (Rompun-Cutter Laboratories). To obtain estimates of initial anesthetic concentrations, the following sources were consulted: BELL (1964), RUNHAM *et al.* (1965), and the manufacturers' literature.

At ambient seawater temperature, anesthesia with chemicals was conducted on an expanded animal placed in one liter of seawater in a glass dish of 20 cm diameter and 7 cm height. Liquid chemicals then were added slowly to the bath, with mixing accomplished by a peristaltic pump. Carbon dioxide simply was bubbled into the bath.

The behavior of an animal was visually monitored during the induction. Generally the signs noted were as follows: (1) hyperactivity, unusual crawling or extreme expansion of the foot and mantle; (2) retraction responses, either of the whole or parts of the animal; (3) lack of responsiveness to a water jet and touch of a stainless steel probe; and (4) flaccid expansion, especially of the tentacles.

If induction was successful the animal was allowed to recover in running seawater, after which the amount of anesthetic was calculated. Each chemical was given six individual animal trials; for the successful ones a second phase of testing occurred in an attempt to establish the minimum concentration needed. This was accomplished by testing at one half the initial concentration and then again at added 10% increments.

After establishing the minimum concentration, a new

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Table 1

Summary of anesthetic actions on the moon snail *Polinices lewisii.*

Anesthetic	No. of ani-mals	Concentration (%)	Concentration (μg/mL)	Induc-tion time (h)	% re-tracted during sur-gery[1]	% sur-vival[2] after 1 week
Althesin	18	0.15	14[3]	2	22	93
Benzyl alcohol	10	0.5–1	—	>1	0	10
Ethanol	15	4	—	3	47	75
Sodium pento-barbital	10	0.14	91	2.5	20	38
Xylazine	20	0.06	12	2.5	35	62
Cold (2°C)	8	—	—	1.5	50	100

[1] Surgery lasted 0.5 h.

[2] Retracted animals eliminated.

[3] This is the final concentration of alphaxalone, one of two active ingredients in Althesin. The commercially available preparation of Althesin contains alphaxalone (9 mg/mL) and alphadolone acetate (3 mg/mL). The latter compound, although possessing some anesthetic activity in humans, is added to increase the solubility of the mixture (REYNOLDS & PRASAD, 1982).

batch of animals subjected to the successful anesthetics underwent surgery. This operation consisted of cutting a hole of 0.5-mm diameter in the dorsal part of the shell and another of 4-mm diameter in the left ventral part of the shell in order to implant impedance-conversion electrodes. A Biocom impedance converter coupled to a Gould recorder was used to make recordings of body movements during the early recovery phase; total recovery of these animals was monitored for at least one week.

RESULTS

For various reasons the following substances were rejected as anesthetics for moon snails: carbon dioxide (up to saturation), magnesium chloride (2.4%), MS 222 (1 mg/mL) and quinaldine (saturated solution). Carbon dioxide and magnesium chloride both failed to induce anesthesia within four hours. However, MS 222 and quinaldine appeared to be highly noxious and caused the moon snails to retract into the shell. Once retracted the moon snails did not extend while immersed in the solutions containing MS 222 and quinaldine.

All other chemicals and hypothermia induced varying states of anesthesia. Table 1 summarizes the results of the second-phase testing of those agents. Exactly when the effects of the agents wore off was difficult to ascertain; therefore, the percentage of animals that retracted during surgery was used as the comparative indicator of that factor.

The behavior of animals during recovery was variable and depended on the anesthetic used. Moon snails that were recovering from cold exposure and ethanol immersion remained quiet for one to two hours after being placed in running seawater, after which the animals tended to crawl around the recovery tank for several hours. However, with Althesin, benzyl alcohol, and xylazine, the quiescent period was more extensive and lasted as long as a day. During the longer quiescent periods many of the animals turned upside down, but this behavior was also observed in normal animals both in the wild and in captivity.

Impedance recordings of body movement within the shell revealed that the quiescent period masked other aspects of the recovery (Figure 1). In all instances body movements

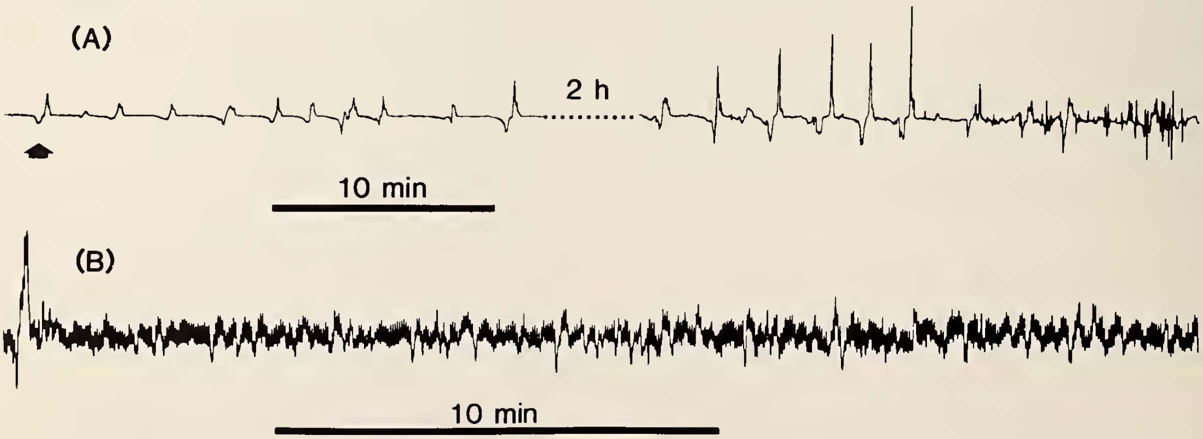

Figure 1

Impedance recording of body movements within the shell of a specimen of *Polinices lewisii* following Althesin anesthesia. (A): early recovery period—the arrow indicates the onset of the body movements, which in this instance occurred approximately 1.2 h after the animal was placed in running seawater to recover. (B): fully recovered animal—the recording was made while the animal was crawling and occurred 40.7 h after the animal was in running seawater. Unlike the previous recording (A), the predominant activity in (B) is the beating of the heart upon which is superimposed other body movements.

occurred within the shell with no apparent external body conformational changes. The earliest onset of these internal body movements followed cold exposure and Althesin immersion, with benzyl alcohol delaying the movements longest.

Table 1 also demonstrates that benzyl alcohol was the most lethal of the chemicals, inducing such visible signs of distress as the sloughing off of an animal's epidermis and pigment within two days. Deaths from the other agents gave no such outward indication.

DISCUSSION

The results of this study demonstrate that anesthesia of the moon snail *Polinices lewisii* can be accomplished by immersion in solutions of anesthetic agents. Because a primary requirement of the study was to ensure that animals remained expanded, no attempt was made to inject anesthetics. In this regard, there was also a practical consideration of finding a blood space within the voluminous aquiferous system, for BERNARD (1968) has shown that system to contain a volume of water equal to 50% of the total weight.

This study also revealed several well-known aquatic vertebrate anesthetics, carbon dioxide, MS 222, and quinaldine (BELL, 1964), to be ineffective for moon snails. For MS 222 and quinaldine the problem appeared to be their highly noxious nature, which caused animals to retract into the shell. However, rejection of carbon dioxide was based on its failure to induce anesthesia after four hours.

Like carbon dioxide, magnesium chloride was also rejected for its inability to cause anesthesia within a reasonable time. In the Mollusca, as in many invertebrates, magnesium chloride is most widely used as a narcotizing agent (RUNHAM *et al.*, 1965). Runham and his colleagues, employing magnesium chloride to anesthetize the marine prosobranchs *Littorina littorea* and *Nucella lapillus,* found that it took overnight to accomplish the task.

Hypothermia appears to be a reasonable means of anesthesia when the surgical procedures are simple. ANDREWS & TANSEY (1981) indicated that hypothermia is a desirable method in *Octopus,* especially for neuropharmacological experiments.

Of the several chemicals, Althesin appears to be the optimal agent for use in the moon snail. It possesses several acceptable characteristics: low lethality, comparatively short induction time, and relatively quick recovery time. Alphaxalone, the major active ingredient of Althesin, has been injected into the crabs *Carcinus maenas* and *Cancer pagurus* to bring about a "sleep time" of two to three hours (OSWALD, 1977). In the moon snail the "sleep time" following Althesin anesthesia appears to be similar to that of crabs subjected to alphaxalone.

Xylazine ranks as the second chemical of choice, possessing a higher lethality than Althesin. OSWALD (1977) found xylazine to be a desirable, fast-acting anesthetic for crabs.

Ethanol and sodium pentobarbital could be used, but have little to offer in preference to Althesin and xylazine. Obviously if cost and availability are factors, then ethanol can be considered. The lethal nature of benzyl alcohol makes it wholly undesirable as an anesthetic in moon snails.

No attempt was made in this study to elucidate the mode of action of any of the chemicals. It is tempting to speculate that the drugs Althesin and xylazine were exerting a central action. However, more study is needed to confirm this as well as to understand the site of entry, distribution, and breakdown of these drugs. Althesin and xylazine should be tested in other gastropod species to see whether they may serve as general anesthetics for snails.

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